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STEREOSELECTIVE SYNTHESIS OF (R)-PROPANE-1, 2-DIOL USING KETOREDUCTASE ENZYMES AND ITS COMPARATIVE STUDIES USING PLUG FLOW REACTOR

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ABSTRACT

A novel and easy enzymatic process for the stereoselective reduction of 1-Hydroxypropan-2-one for the synthesis of (R)-propane-1, 2-diol (Scheme-1) was identified. (R)-propane-1, 2-diol can serve as a key intermediate of many pharmaceutically active compounds. Single step conversion was achieved using Ketoreductase enzymes with high enantioselectivity and better yield in comparison with chemical process. 100% chiral purity has been obtained for the enantiomerically pure intermediate, confirmed by both TLC and Chiral HPLC.

KEYWORDS

Ketoreductase enzymes, 1-Hydroxypropan-2-one, (R)-propane-1, 2-diol and Chiral purity.

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INTRODUCTION

Most of the drugs currently in use are chiral compounds[1] and marketed as racemates consisting of an equimolar mixture of two enantiomers. They have the same chemical structure, most isomers of chiral drugs exhibit significant differences in biological activities[2] such as pharmacology, toxicology, pharmacokinetics, metabolism etc. Therefore, it is important to promote the chiral synthesis of racemic drugs in pharmaceutical industry as well as in clinic in order to eliminate the unwanted isomer from the preparation and to find an optimal treatment and a right therapeutic control for the patient.

Bio catalysis[3] is defined as the use of biomolecules, especially enzymes as catalysts for the synthesis of new materials. Enzymes[4] can be thought of as renewable catalysts. Unlike metal-based alternatives that rely on mining and harsh, energy intensive processing, biocatalysts are biodegradable and easily replaced through inexpensive and environmentally benign fermentation processes. Some of the most powerful examples of biocatalytic applications exploit the chiral nature of these catalysts. In fact, the *R* and *S* nomenclature commonly used to define the orientation of substituents of a chiral molecule.

Flow chemistry[5] is defined as a chemical reaction running continuously through a flowing stream rather than in batch production. Pumps move fluid into a tube and the fluids contact each other to reaction taken place. Nowadays flow chemistry have been applied in bio catalysis as well. R-propane 1, 2 diol is a protic solvent that can act as proton or hydrogen donor during chemical reactions. R-propane 1, 2 diol is a clear, colorless, viscous organic solvent and diluent[6] used in pharmaceutical preparations.

Ketoreductase[7] catalyze the conversion of keto group to a chiral hydroxyl group, Ketoreductases are highly enantioselective, so they can be used for synthesizing chiral hydroxyl compounds from inexpensive ketones. The ketone compound can be directly converted tohydroxy for the formation of required (R)-propane-1, 2-diol compound in a single step with better enantiomeric purity than the chemical process.

By using bio catalysis technique and Ketoreductase, we have converted the 1-Hydroxypropan-2-one to (R)-Propane-1, 2-diol in presence of potassium phosphate buffer and environmentally friendly conditions.

MATERIAL AND METHODS

Reagents and chemicals

In the experimental section, unless and otherwise stated, all reagents and solvents used in this study are commercially obtained. Ketoreductases were purchased from Iosynth, Bangalore, India and Enzyme works, China.

Methodology

Experimental Section using batch reactor

Step I: Preparation of Buffer Solution

In a flask, Disodium Hydrogen Phosphate (1.5g), water (100ml) and charge Dihydrogen Sodium Ortho Phosphate (1.0g). Stir for clear solution and add Isopropyl alcohol 5ml (pH-7.0-7.5).

Step II: Preparation of (R)-Propane-1, 2-diol

To the buffer solution (3.0ml) was added Ketoreductase enzyme (50mg) and 1-Hydroxypropan-2-one (5.0mg). Temperature was raised to 25°C and maintained at same for 120 hours. Reaction was monitored by TLC. After completion of the reaction filtered the reaction mass through hiflo, to the reaction mixture added10% sodium hydroxide solution (0.2ml) and stirred for 30 mins at 20-25°C. The resulting reaction mass was extracted with ethyl acetate and dried over anhydrous Sodium Sulfate and distilled under vacuum to yield (R)-Propane-1, 2-diol (1.8mg).

Experimental procedure using plug flow reactor

Step I: Preparation of buffer solution

In a flask, Disodium Hydrogen Phosphate (1.5g), water (100ml) and charge Dihydrogen Sodium Ortho Phosphate (1.0g). Stir for clear solution and add Isopropyl alcohol 5ml (pH-7.0-7.5).

Step II: Preparation of (R)-Propane-1, 2-diol

To the buffer solution (25.0ml) was added Ketoreductase enzyme (4mg) and 1-Hydroxypropan-2-one (20.0mg). Pass the reaction mixture into flow reactor through flow pump at 40°C for 27hours. At flow rate 0.2ml/min. Reaction was monitored by TLC, after completion of the reaction, filtered the reaction mass through hiflo to the reaction mixture added 10% sodium hydroxide solution (0.2ml) and stirred for 30 mins at 20-25°C. The resulting reaction mass was extracted with ethyl acetate and dried over anhydrous Sodium Sulfate and distilled under vacuum to yield (R)-Propane-1, 2-diol (15.8mg).

TLC Conditions

To the TLC plate, were applied spots of our keto compound and final product which was immersed in a mobile phase of following composition i.e., Dichloromethane: Methanol = 9:1 respectively. The

plate was then viewed after sprayed with Potassium permanganate solution.

RESULTS AND DISCUSSION

Several variants of Ketoreductase enzymes were screened in the following study out of which two variants showed significant product formation and the results were observed as follows in Table No.1 and Table No.2.

Product conversion observed by TLC for our required (R)-Propane-1, 2-diol compound as shown below (Figure No.1) for the mobile phase conditions MDC: Methanol = 9:1 previously described.

The chiral HPLC data in column chiral pack1G (250 x 4.6) mm 5µm for the intermediate prepared through synthetic route is as follows mentioned in Figure No.2.

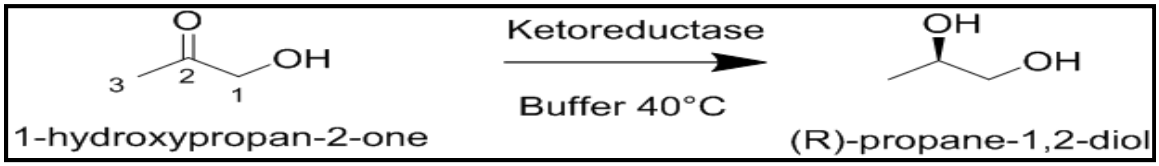
For 100% Chiral purity in column chiral pack 1G(250 x 4.6) mm 5µm for the desired (R)-Propane-1, 2-diol compound prepared using ketoreductase enzymes, the Chiral HPLC data is as follows mentioned in Figure No.3.

Table No.1: Synthesis of (R)-propane-1, 2-diol

S.No	Enzyme Variant	PRODUCT	Time taken for reaction completion	Chiral Purity	Enzyme Loading with respect to 1-Hydroxy propane-2-one
1	CN102-KRED-LP090	(R)-propane-1, 2-diol	120 HRS	100%	10.0 Times
2	CN102-KRED-LP095	(R)-propane-1, 2-diol	120 HRS	100%	10.0 Times
3	CN102-KRED-LP092	(R)-propane-1, 2-diol	120 HRS	51.53%	10.0 Times
4	CN102-KRED-LP096	(R)-propane-1, 2-diol	120 HRS	48.63%	10.0 Times
5	CN102-KRED-LP100	(R)-propane-1, 2-diol	120 HRS	51.73%	10.0 Times
6	CN102-KRED-LP097	(R)-propane-1, 2-diol	120 HRS	48.42%	10.0 Times

Table No.2: Comparison of Ketoreductase reaction in Batch reactor and plug flow reactor

S.No	Enzyme Variant	Reactor	PRODUCT	Time taken for reaction completion	Chiral Purity	Enzyme Loading with respect to 1-Hydroxy propane-2-one
1	CN102-KRED-LP090	Batch reactor	(R)-propane-1, 2-diol	120 HRS	100%	10 TIMES
2	CN102-KRED-LP090	Flow reactor	(R)-propane-1, 2-diol	27 HRS	99.03	0.2 TIMES
3	CN102-KRED-LP095	Flow reactor	(R)-propane-1, 2-diol	27 HRS	99.05	0.2 TIMES
4	EW-KRED-R118	Flow reactor	(R)-propane-1, 2-diol	27 HRS	99.07	0.2 TIMES
5	EW-KRED-R144	Flow reactor	(R)-propane-1, 2-diol	27 HRS	99.07	0.2 TIMES
6	EW-KRED-R104	Flow reactor	(R)-propane-1, 2-diol	27 HRS	99.04	0.2 TIMES
7	CN102-KRED-LP092	Flow reactor	(R)-propane-1, 2-diol	27 HRS	50.31	0.2 TIMES
8	CN102-KRED-LP096	Flow reactor	(R)-propane-1, 2-diol	27 HRS	50.35	0.2 TIMES
9	CN102-KRED-LP100	Flow reactor	(R)-propane-1, 2-diol	27 HRS	50.34	0.2 TIMES
10	CN102-KRED-LP097	Flow reactor	(R)-propane-1, 2-diol	27 HRS	50.45	0.2 TIMES



Scheme No.1: For the synthesis of (R)-2-propane-1, 2-ol

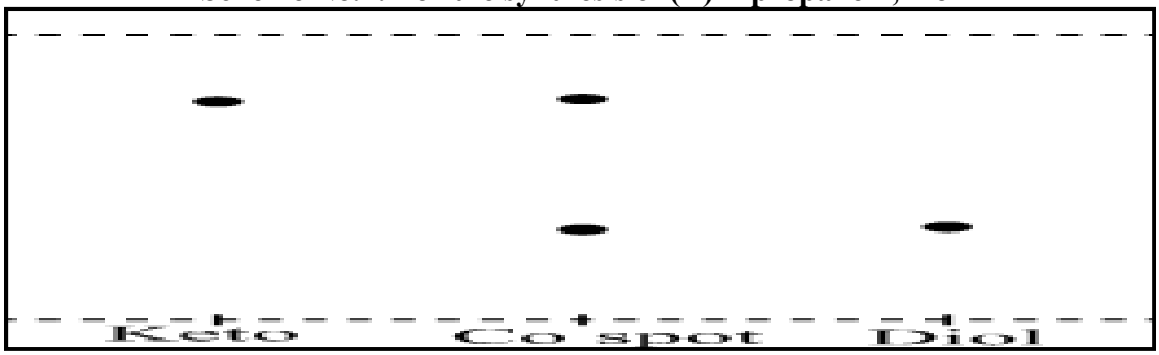


Figure No.1: Thin layer chromatograph of reaction monitoring

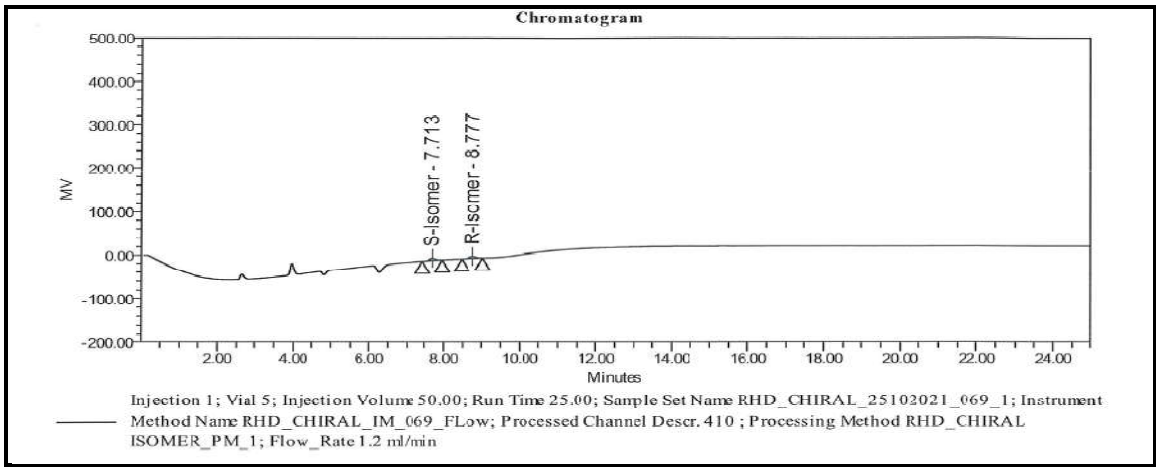


Figure No.2: Chiral HPLC chromatogram for the intermediate

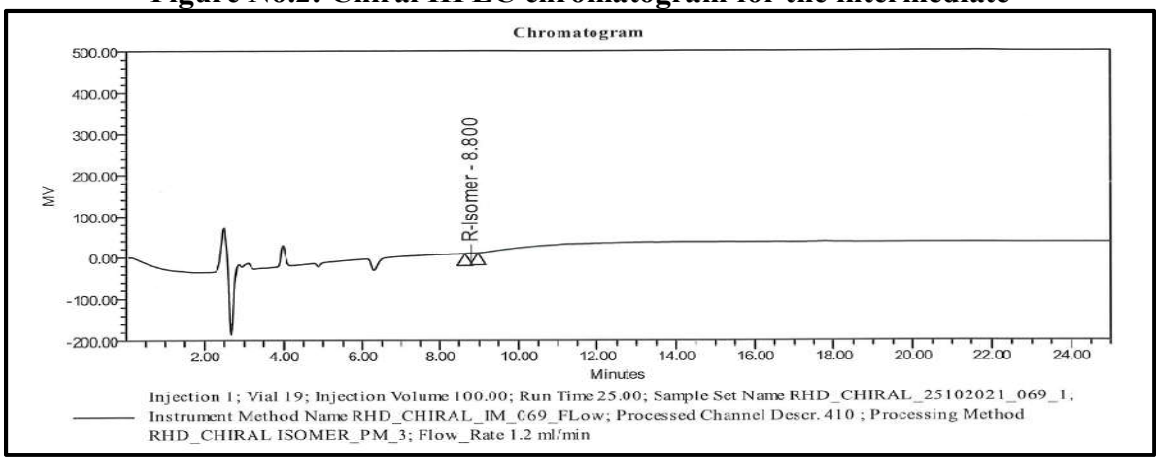


Figure No.3: Chiral HPLC chromatogram for the chiral purity for (R)-Propane-1, 2-diol

CONCLUSION

It can be determined from the above study that, (R)-Propane-1, 2-diol which can serve as key chiral intermediate of the many active pharmaceutical ingredients. (R)-Propane-1, 2-diol can be prepared using Ketoreductase enzyme with 100% chiral purity in a single step opposed to conventional chemical processes involving multiple steps with poor yields and low enantioselectivity. We have reduced reaction time hours to 27 hours and Enzyme loading to 0.2 times using flow reactor as compared to batch reactor in which reaction time hours is 120 hours and Enzyme loading is higher side i.e.,10.0 times with respect to 1-Hydroxy propane-2-one.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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